

WHAT IS CLAIMED IS:

1. A method of identifying the location of a compound in an assay pattern created in a free-form biological assay, comprising:

providing an image of the assay pattern, wherein the image has pixels that depict a spot;

identifying the center of the spot by analyzing a plurality of pixels in the image;

generating a model of a signal at the location of the spot, wherein the model of the signal is based on the diffusion of a reactive compound in a reagent containing layer;

determining whether the spot is a signal by comparing the spot and the model; and

for a spot identified as a signal, determining the sample compound location on the assay pattern that corresponds to the image location of the center of the spot.

2. The method of Claim 1, wherein generating a model of the signal comprises generating a parametric model of the signal.

3. The method of Claim 2, wherein generating a parametric model of the signal comprises:

generating a plurality of parameters describing the spot depicted in the image; and

generating a model of the signal using the parameters.

4. The method of Claim 3, wherein comparing the spot and the model comprises: generating a correlation value that provides a measure of fitness between the model and the parameters, and

determining whether the correlation value exceeds a threshold value.

5. The method of Claim 3, further comprising regenerating the parameters of the spot and regenerating the correlation value such that the regenerated parameters affect an increase in the regenerated correlation value.

6. The method of Claim 1, wherein analyzing a plurality of pixels in the image comprises interactively identifying a candidate signal from a displayed digital image.

7. The method of Claim 1, wherein analyzing a plurality of pixels in the image comprises identifying a candidate signal location using automatic image processing.

8. The method of Claim 7, wherein the image processing comprises calculating a pixel intensity slope for each pixel in a set of pixels, storing the results of the calculating step, and combining the stored results to identify the location of the signal.

9. A method of identifying the location of a signal in an image of a biological assay, comprising:

providing an image of the assay, wherein the image has a plurality of pixels depicting the signal;

defining a subimage pixel area in the image;

centering the subimage pixel area on a target pixel in the digital image;

calculating a pixel intensity slope for the target pixel, wherein pixels contained within the subimage area are used to calculate the pixel intensity slope of the target pixel;

storing the result of the calculating step;

repeating the centering, calculating, and storing steps for a plurality of target pixels in the digital image; and

combining the stored results to identify the location of the signal.

10. The method of Claim 9, further comprising providing a transform image having pixels, wherein target pixels in the digital image each have a corresponding pixel in the transform image, and wherein the stored results are combined in the transform image.

11. The method of Claim 9, wherein a threshold value is applied to the combined results, and wherein spot locations are identified by a combined result that exceeds the threshold value.

12. The method of Claim 10, wherein calculating the pixel intensity slope comprises:

assigning to a target pixel one or more values representative of the intensity or color of the target pixel;

determining one or more values for neighbor pixels around the target pixel; and

if the value assigned to the target pixel is different from values of the neighbor pixels, determining a direction representative of maximum change or rate of change of the value from the target pixel into the neighbor pixels, and associating a vector with the target pixel indicative of the direction.

13. A method for identifying a hit spot in a free-form biological assay, where the hit spot is the result of an interaction between a sample compound and a reactive agent, comprising:

providing a digital image, wherein the image depicts a plurality of candidate spots which may include a hit spot;

analyzing the image by image processing means to identify a first candidate spot;

generating a spot function parametrically modeling the first candidate spot; and

analyzing the spot function and the first candidate spot to identify a hit spot depicted in the digital image.

14. The method of Claim 13, further comprising:

correlating the first candidate spot to a replicate spot depicted in the image;

generating a spot function parametrically modeling the replicate spot; and

wherein analyzing further comprises analyzing the spot function of the replicate spot and the replicate spot to identify the hit spot depicted in the image.

15. The method of Claim 13, further comprising generating a spot correlation value, the correlation value providing a measure of fitness between the spot function and the first candidate spot, and wherein analyzing further comprises analyzing the spot correlation value.

16. The method of Claim 15, further comprising:

generating candidate spot parameters describing the first candidate spot; and

wherein analyzing further comprises analyzing the first candidate spot parameters.

17. The method of Claim 16, wherein parameters comprise radius and amplitude.

18. The method of Claim 17, wherein parameters further comprise sigma, base, flatness, and a flatness threshold.

19. The method of Claim 14, further comprising:

generating a replicate spot correlation value, the replicate spot correlation value providing a measure of fitness between the replicate spot function and the replicate spot; and

wherein analyzing further comprises analyzing the replicate spot correlation value.

20. The method of Claim 19, further comprising:
generating replicate spot parameters describing the replicate spot depicted in the image; and

wherein analyzing further comprises analyzing the replicate spot parameters.

21. A system for identifying a signal location in a digital image of a biological assay, comprising:

a gradient triangulation subsystem with means for identifying the location of a candidate signal in the image; and

a signal modeling subsystem with means for processing a set of pixels in the image proximate to the candidate signal location to determine if a signal exists at the candidate signal location.

22. The system of Claim 21, further comprising an alignment subsystem with means for identifying a plurality of alignment spots depicted in the image and matching the alignment spots to a known alignment pattern.

23. The system of Claim 21, further comprising a preprocessing subsystem configured to filter noise from the image.

24. A method of identifying a hit spot depicted in an image, comprising:
providing a digital image, wherein the image may depict hit spots;
processing the image by image processing means to acquire a set of spots depicted in the image;

generating parameters for each spot in the set;

generating a spot function for each spot in the set, the spot function parametrically modeling each spot; and

analyzing the spot function and the parameters to identify hit spots from the set of spots depicted in the image.

25. The method of Claim 24, further comprising:
generating a correlation value for each spot in the set of spots, the correlation value providing a measure of fitness between the spot function and each spot, and

wherein said analyzing further comprises analyzing the correlation values.

26. The method of Claim 25, further comprising:
generating a list of spots having a high correlation value;

for the list of spots:

- (a) optimizing the parameters of a selected spot on the list, the selected spot having the highest value;
- (b) removing the selected spot from the list of spots;
- (c) removing information related to the selected spot from the image;
- (d) generating a new correlation value for each spot remaining on the list; and
- (e) repeating steps (a) – (d) until there are no remaining spots on the list.

27. A method of correlating a hit spot depicted in an image with a corresponding sample compound location, comprising:

providing a digital image, wherein the digital image depicts one or more alignment spots and may depict hit spots;

identifying one or more alignment spots depicted in the image;

registering the image by matching the one or more alignment spots to a known alignment pattern;

identifying a spot depicted in the image;

generating a spot function, the spot function parametrically modeling the spot;

comparing the spot function and the spot to determine if the spot is a hit spot; and

correlating the location of the hit spot depicted in the image with a known sample compound pattern to identify a sample compound location corresponding to the location of the hit spot.

28. The method of Claim 27, wherein matching comprises manually matching.

29. The method of Claim 27, wherein matching comprises matching using image processing means.

30. The method of Claim 29, wherein the image processing means comprises gradient triangulation.

31. The method of Claim 27, wherein registering the image further comprises generating a theta value, wherein theta is an alignment factor for rotational correction.

32. The method of Claim 31, wherein registering the image further comprises generating at least one scale, wherein the scale factor is an alignment factor for converting an image measurement to a distance measurement.

33. The method of Claim 32, wherein the scale factor is used in computing the conversion from image pixels to millimeters.

34. The method of Claim 32, wherein registering the image further comprises computing at least one offset factor, wherein the offset factor is used in computing the true position of an alignment spot.

35. A method of correlating a signal in a representative digital image of a free-form biological assay to an associated sample compound location, comprising:

identifying the location of a candidate signal in the digital image;

generating a function to model a signal formed in a free-form biological assay;

generating a parameter describing the candidate signal;

generating a correlation value, the correlation value being a measure of fitness between the function and the candidate signal;

analyzing the digital image using the correlation value to identify a signal location in the digital image; and

correlating the signal location with a known assay pattern to identify a sample compound location.

36. A computer readable medium tangibly embodying a program of instructions executable by a computer to perform a method of identifying a location in of a sample compound that generated a hit spot in a biological assay, the method comprising:

providing a digital image of the assay, wherein the image comprises pixels depicting a spot;

analyzing the pixels to identify the location of the spot;

generating a parameter describing the spot;

generating a spot function using the parameter, the spot function parametrically modeling the spot;

generating a correlation value, the correlation value being a measure of fitness between the spot function and the spot;

analyzing the correlation value to determine if the spot is a hit spot; and

matching the location of the hit spot in the image with an assay pattern to identify a sample compound location.

37. A method for identifying features of an image, comprising:
providing a digital image comprising pixels;
for a set of pixels in the image:
- (a) assigning to a target pixel one or more values representative of one or more of intensity or color of the target pixel;
 - (b) determining the one or more values for neighbor pixels around the target pixel;
 - (c) if the value assigned to the target pixel is different from values of the neighbor pixels, determining a direction representative of maximum change or rate of change of the value from the target pixel into the neighbor pixels, and associating a vector with the target pixel indicative of the direction;
 - (d) repeating steps (a)-(c) for each pixel in the set; and
 - (e) identifying one or more features by identifying a pattern from said vectors.
38. The method of Claim 37, wherein pattern comprises intersection of vectors.
39. The method of Claim 37, further comprising graphically representing vectors as symbols in a visual image.
40. The method of Claim 37, wherein the symbols represent the direction of the vectors.
41. The method of Claim 37, wherein the symbols represent the direction and magnitude the vectors.
42. The method of Claim 37, further comprising preparing a data set comprising the vectors generated in steps (a)-(d).
43. The method of Claim 42, wherein data set includes coordinates associated with each vector.
44. A method for identifying the location of a spot in an image of a multiplexed assay, comprising:
- selecting a first target location in said image;
 - comparing the color or intensity of the first target location with that of surrounding target locations to ascertain a direction of a maximum color or intensity change through said first target location, referred to herein as an intensity slope vector;

repeating the selecting and comparing steps with other target locations in said image to identify a location in said image where intensity slope vectors converge.

45. The method of Claim 44, wherein said target locations are pixels.

46. The method of Claim 44, further comprising correlating the location of the spot to the identity or location of a compound dot used in said multiplexed assay.

47. A method of registering a digital image of a biological assay, comprising:
providing a digital image containing pixels, wherein the pixels depicts a plurality of spots;

identifying one or more alignment spots depicted in the image;

matching the one or more alignment spots to a known pattern of alignment spots;

calculating a plurality of alignment factors for a plurality of locations in the image based on said matching; and

registering the image using the alignment factors to match the spot locations to known locations using a sample compound pattern.

48. The method of Claim 47, where the alignment factors are calculated for every pixel in the digital image.

49. The method of Claim 47, where the alignment factors comprise (x, y) offset.

50. The method of Claim 47, where the alignment factors comprise (x,y) scale.

51. The method of Claim 47, where the alignment factors comprise theta rotation.

52. A method of registering a digital image to identify a hit spot in an image with a corresponding sample compound location, comprising:

providing a digital image, wherein the digital image depicts a plurality of alignment spots and at least one pair of hit spots;

identifying one or more alignment spots depicted in the image;

registering the image by matching the one or more alignment spots to a known alignment pattern;

identifying a probable pair of hit spots depicted in the image;

calculating a plurality of alignment factors using the locations of the probable pair of hit spots and the alignment spots, and using known patterns of pairs of hit spots and the alignment patterns;

registering the image using the calculated alignment factors to match the locations of the image to known locations in a sample compound pattern; and

determining if an additional probable pair of hit spots is in the image, and if so, iteratively repeating said calculating step and said registering step using the additionally identified pair of hit spots.

53. A method of identifying a hit spot in an image, comprising:
providing a digital image, wherein the image may depict hit spots;
processing the image by image processing means to acquire a set of spots depicted in the image;

generating parameters for each spot in the set;

generating a value for each spot in the set, wherein the value is a measure of whether the spot is a hit spot;

generating a list of spots having a high value;

for the list of spots:

(a) optimizing the parameters of a selected spot on the list, the selected spot having the highest value;

(b) removing the selected spot from the list of spots;

(c) removing information related to the selected spot from the image;

(d) generating a new value for each spot remaining on the list;

(e) repeating steps (a) – (d) until there are no remaining spots on the list;

and

analyzing a spot using its value to identify the spot as a hit a spot.

54. The method of Claim 53, wherein the value relates to the intensity of the spot depicted in the digital image.

55. The method of Claim 53, wherein the value relates to the size of the spot depicted in the digital image.